

B Cell Diversification and Differentiation in the Periphery

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Humoral responses to thymus-dependent antigens begin in the T cell zones of secondary lymphoid tissues (1–5) where antigen-activated T and B lymphocytes make physical contact (6). Though both swift and brief (4, 7), this encounter initiates a complex program of B cell differentiation leading to specialization for early antibody production or immune memory. In the spleen, B cells initially proliferate in the T cell-rich periarteriolar lymphoid sheath (PALS) and then either develop locally into foci of antibody-secreting cells or migrate to the nearby lymphoid follicle to initiate germinal center (GC) formation (3–5).

In the follicle, these antigen-specific B cell immigrants interact with follicular dendritic cells (FDC) that are specialized for the retention and presentation of unprocessed antigen complexed with antibody (8, 9). This interaction results in further rounds of B cell proliferation within the FDC reticulum and leads to the generation of a GC (3, 4). GCs are finely structured in some species, containing a dark zone of rapidly proliferating centroblasts proximal to the PALS and more distal basal and apical light zones containing nondividing centrocytes (4). The light zones also contain the densest region of the FDC network and the few (5–10% of GC cells) T lymphocytes found in GCs (10) (Fig. 1). Interestingly, recent work (11, 12) has suggested that these T cells are antigen-specific and selectively recruited into the GC.

GCs are necessary for the generation of the B cell memory compartment (13) and are the site of Ig V-region hypermutation required for the affinity maturation of serum antibody (14–17). Typically, V-region mutations appear at about day 7 of the primary response, coincident with the end of the initial proliferative phase of the GC reaction and at the formation of the light and dark zones. Mutations are introduced in a stepwise manner for at least 14 d and the kinds and distribution of Ig mutations suggest that phenotypic selection also occurs within the GC microenvironment (14, 16–18). This selection generally favors higher affinity mutants and is believed to be driven by competition for antigen displayed on the FDC surface. GC B cells that survive repeated rounds of mutation and selection enter the memory cell compartment and dominate later responses (18).

The signals that mediate these processes of proliferation, migration, mutation, and differentiation are not known in detail; however, several recent studies have provided a glimpse of the molecular basis for these events. Genetic defects (19) or the early administration of antibodies (6) or fusion proteins (20) that prevent cognate (CD40:CD40L) or costimula-

tory (B7:CD28/CTLA-4) interactions between B and T lymphocytes block the GC reaction. It remains unclear if these agents inhibit the initial T-B interaction in the PALS or if they act after the GC has been formed.

In this issue of *The Journal of Experimental Medicine*, Virginia Pascual and her colleagues in Dardilly and Dallas demonstrate that multiparameter flow cytometry may be used to identify five developmental compartments within populations of human tonsillar lymphocytes (21). This tissue is rich in GCs and provides the basis for much of our knowledge about this remarkable microenvironment. Unfortunately, GCs are constitutively present in the tonsil, making it difficult to discern the temporal order of developmental events (22). Pascual et al. have neatly resolved this issue by convincingly defining a developmental series that includes, naive, GC, and memory B lymphocytes. Naive B cells are defined as small dense lymphocytes expressing high levels of surface IgD and

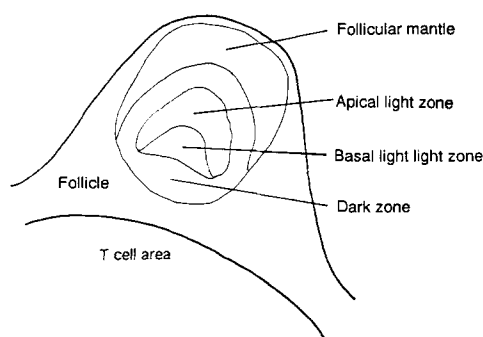


Figure 1. A generalized GC (after reference 10) illustrating the cellular compartments isolated by Pascual et al. (21). After activation through contact-dependent mechanisms in the T cell areas, e.g., the PALS of spleen, antigen-specific T and B lymphocytes migrate into the lymphoid follicle. There, B cells begin to proliferate within the FDC reticulum forming the nascent GC. After a period of intense proliferation, the dark and light zones are formed and Ig hypermutation commences. sIgD⁺ B lymphocytes (Bm1 and Bm2 cell fractions) enter the GC and there become sIgD⁻ and acquire CD38. CD38⁺ cells may be identified as dark zone centroblasts (CD77⁺; Bm3) or light zone centrocytes (CD77⁻; Bm4). It is believed that V-region mutations are acquired by the proliferating centroblasts whereas mutant centrocytes are selected for their ability to bind antigen displayed by the FDC within the apical light zone; GC B cells express very low levels of the antiapoptotic protein Bcl-2 and die in the absence of rescuing signals mediated by sIg and the CD40 molecule. Recirculating, memory B lymphocytes (Bm5) lose CD38 expression and regain cytoplasmic Bcl-2. These cells are found in the follicular mantle.

IgM. These cells contain abundant Bcl-2 protein (23) in their cytoplasm, and are CD44⁺ and CD77⁻. This compartment is divided by the expression of CD23 (defined by the authors as the Bm2 fraction of cells) or its absence (Bm1 cell fraction). The CD38⁺ GC B cells lose surface IgD and cytoplasmic Bcl-2 but gain expression of the Ki67 antigen, a marker of cellular proliferation. CD38⁺ cells may be identified as centroblasts (CD77⁺; Bm3) or centrocytes (CD77⁻; Bm4). Memory B cells (Bm5) lack IgD, CD38, and the Ki67 marker but regain CD44 expression and cytoplasmic Bcl-2. Molec-

ular genetic analysis of the Bm1–Bm5 fractions indicate that mutation is absent before the Bm3 compartment, consistent with studies of murine responses, and is maintained as selected substitutions in the Bm5 memory cells. Thus, it should now be possible to isolate from tonsil the important cell compartments along the pathway to B cell memory. This approach has proven very successful for studies of B cell development in the bone marrow (24) and will permit investigation of the signals, molecules, and genes that control lymphocyte development and selection within the GC microenvironment.

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References

- Gray, D. 1988. Recruitment of virgin B cells into an immune response is restricted to activation outside of follicles. *Immunology*. 65:73.
- Vonderheide, R.H., and S.V. Hunt. 1990. Immigration of thoracic duct B lymphocytes into established germinal centers in the rat. *Eur. J. Immunol.* 20:79.
- Jacob, J., R. Kassir, and G. Kelsoe. 1991. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl) acetyl. I. The architecture and dynamics of responding cell populations. *J. Exp. Med.* 173:1165.
- Liu, Y.J., J. Zhang, P.J.L. Lane, E.Y.T. Chan, and I.C.M. MacLennan. 1991. Studies of specific B cell activation in primary and secondary responses to T cell-dependent and T cell-independent antigens. *Eur. J. Immunol.* 21:2951.
- Jacob, J., and G. Kelsoe. 1992. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl) acetyl. II. A common clonal origin for periarteriolar lymphoid sheath-associated foci and germinal centers. *J. Exp. Med.* 176:679.
- Van den Eertwegh, A.J.M., R.J. Noelle, M. Roy, D.M. Shepherd, A. Aruffo, J.A. Ledbetter, W.J.A. Boersma, and E. Claassen. 1993. In vivo CD40-gp39 interactions are essential for thymus-dependent humoral immunity. I. In vivo expression of CD40 ligand, cytokines, and antibody production delineates sites of cognate T-B cell interactions. *J. Exp. Med.* 178:1555.
- Van Rooijen, N., E. Claassen, and P. Eikelenboom. 1986. Is there a single differentiation pathway for all antibody forming cells in the spleen? *Immunol. Today*. 7:193.
- Tew, J.G., and T.E. Mandel. 1979. Prolonged antigen half-life in the lymphoid follicles of specially immunized mice. *Immunology*. 37:69.
- Szakai, A.K., M.H. Kosco, and J.G. Tew. 1988. A novel in vivo follicular dendritic cell-dependent iccosome-mediated mechanism for delivery of antigen to antigen processing cells. *J. Immunol.* 140:341.
- Hardie, D.L., G.D. Johnson, M. Khan, and I.C.M. MacLennan. 1993. Quantitative analysis of molecules which distinguish functional compartments within germinal centers. *Eur. J. Immunol.* 23:997.
- Fuller, K.A., O. Kanagawa, and M. Nahm. 1993. T cells within germinal centers are specific for the immunizing antigen. *J. Immunol.* 151:4505.
- Kelsoe, G., and B. Zheng. 1993. Sites of B-cell activation in vivo. *Curr. Opin. Immunol.* 5:418.
- Coico, R.F., B.S. Bhogal, and G.J. Thorbecke. 1983. Relationship of germinal centers in lymphoid tissue to immunologic memory. VI. Transfer of B cell memory with lymph node cells fractionated according to their receptors for peanut agglutinin. *J. Immunol.* 131:2254.
- Berek, C., A. Berger, and M. Apel. 1991. Maturation of the immune response in germinal centers. *Cell*. 67:1121.
- Jacob, J., G. Kelsoe, R. Rajewsky, and U. Weiss. 1991. Intracloonal generation of antibody mutants in germinal centres. *Nature (Lond.)*. 354:389.
- Jacob, J., J. Przylepa, C. Miller, and G. Kelsoe. 1993. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. III. The kinetics of V region mutation and selection in germinal center B cells. *J. Exp. Med.* 178:1293.
- McHeyzer-Williams, M.G., M.J. McLean, P.A. Lalor, and G.J.V. Nossal. 1993. Antigen-driven B cell differentiation in vivo. *J. Exp. Med.* 178:295.
- Weiss, U., and K. Rajewsky. 1990. The repertoire of somatic antibody mutants accumulating in the memory compartment after primary immunization is restricted through affinity maturation and mirrors that expressed in the secondary response. *J. Exp. Med.* 172:1681.
- Korthanuer, U., D. Graf, H. Mages, F. Brière, P. Munoreedevi, S. Malcolm, A.G. Vgazio, L.D. Notarangelo, R.J. Levinski, and R.A. Kroczeck. 1993. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature (Lond.)*. 361:539.
- Lane, P., C. Burdet, S. Hubele, D. Scheidegger, U. Müller, F. McConnell, and M. Kosco-Vilbois. 1994. B cell function in mice transgenic for mCTLA4-Hy1: lack of germinal centers correlated with poor affinity maturation and class switching despite normal priming of CD4⁺ T cells. *J. Exp. Med.* 179:819.
- Pascual, V., Y.-J. Liu, A. Magalski, O. de Bouteiller, J. Banachereau, and J.D. Capra. 1994. Analysis of somatic mutation in B cell subsets of human tonsil correlates with phenotypic differentiation from the naive to the memory B cell compartment. *J. Exp. Med.* 180:319.
- Küppers, R., M. Zhao, M.L. Hansmann, and K. Rajewsky. 1993. Tracing B cell development in human germinal centres by molecular analysis of single cells picked from histological sections. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:4967.
- Vaux, D.L., S. Cory, J.M. Adams. 1988. *bcl-2* gene promotes haemopoietic cell survival and cooperates with *c-myc* to immortalize pre-B cells. *Nature (Lond.)*. 335:440.
- Li, Y.-S., K. Hayakawa, and R.R. Hardy. 1993. The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. *J. Exp. Med.* 178:951.